

Inflammatory response to assess toxicity of biodiesel emission samples

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Biodiesel and Renewable Diesel Multimedia
Evaluation Public Meeting**

Inhalation of environmental and occupational pollutants in vivo

- Pulmonary inflammation, fibrosis, lung carcinomas
- Cardiovascular diseases like atherosclerosis
- Chronic inflammatory response as a main cause for adverse health effects

In vitro cell models

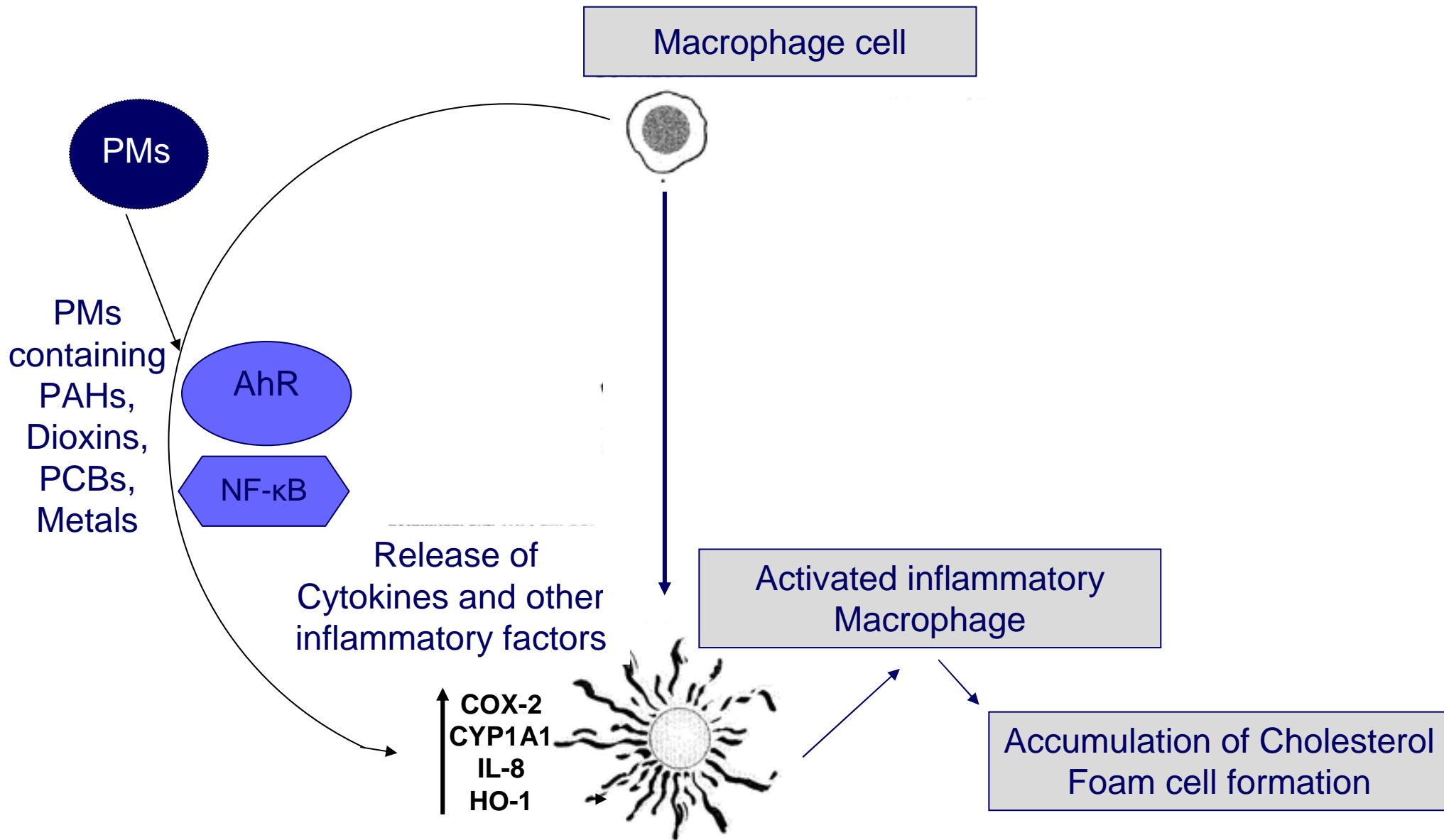
- Two main target cell types
 - a) Macrophages (U937), phagocyte, acts as first line of defense
 - b) Lung Clara cells from pulmonary epithelium (NCI H441)



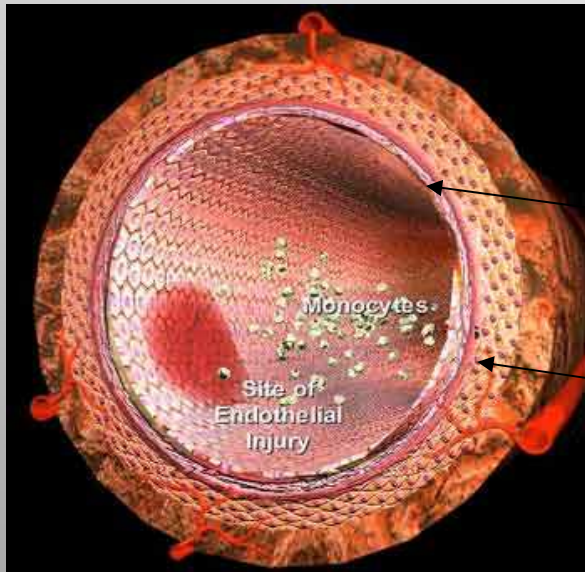
Biomarkers of PM exposure, inflammation and oxidative stress

- CYP1A1: Cytochrome P450 monooxygenase, xenobiotic metabolizing enzyme, Arylhydrocarbon-Receptor regulated
- COX-2: Cyclooxygenase, key enzyme for production of prostaglandins involved in inflammation
- IL-8: Interleukin 8, chemoattractant peptide for neutrophils, major mediator of inflammatory response
- HO-1: Hemeoxygenase 1, essential enzyme in heme catabolism, protect cells against oxidative injury. Induced by exposure to various forms of oxidative stress

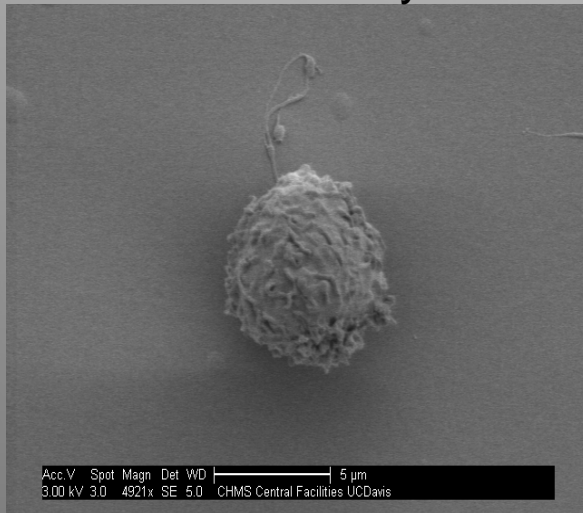
Macrophage Model to measure Inflammation caused by Diesel PM



Formation of foam cells



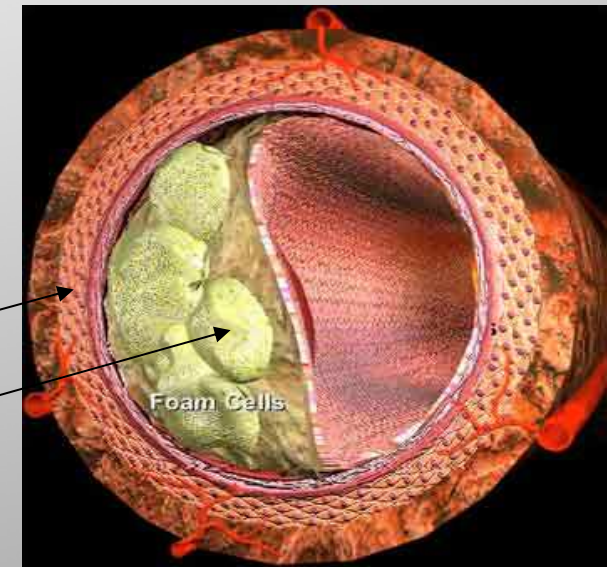
Control monocyte



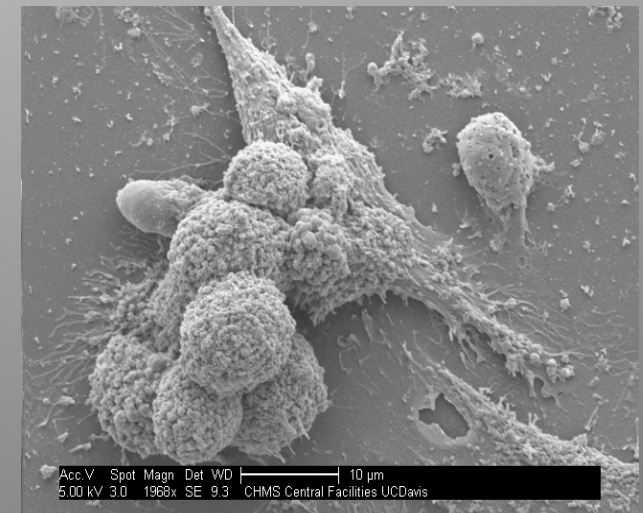
Swelling of the intima in the wall of the artery which pushes the endothelium into the lumen of the artery

Arterial wall

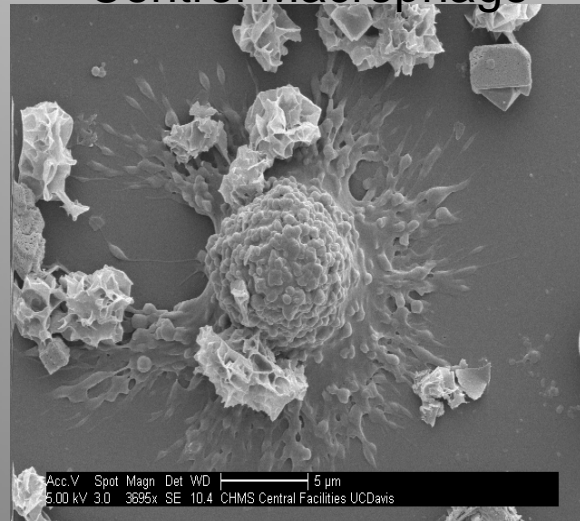
Foam cells



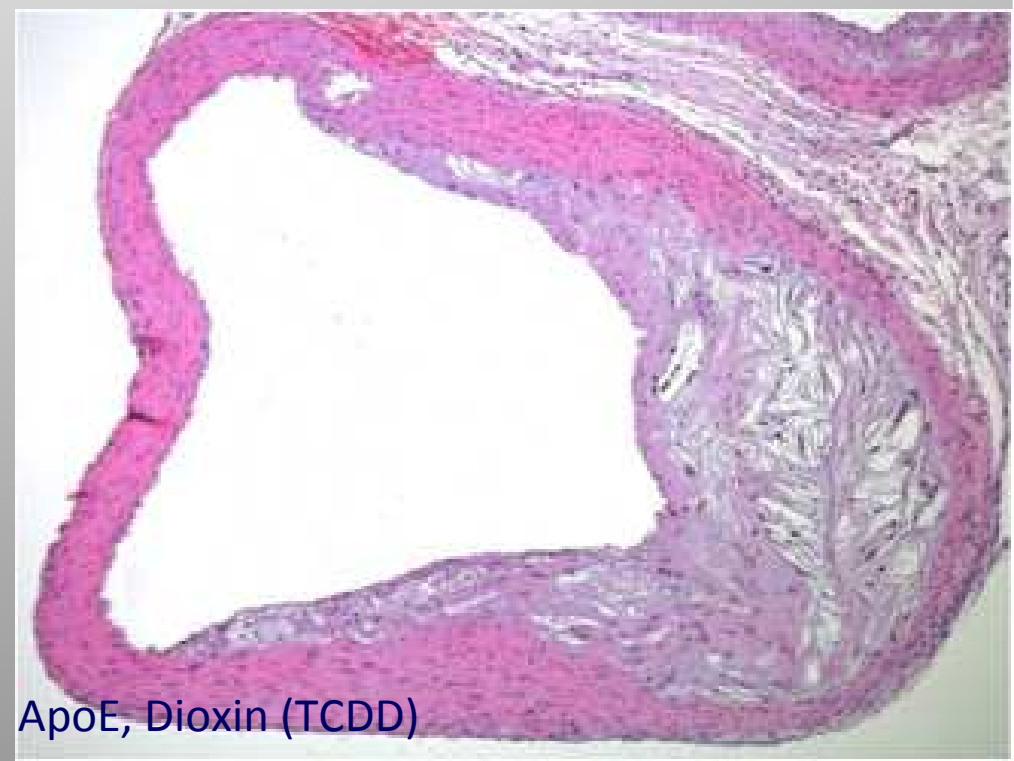
Dioxin Foam Cells



Control Macrophage



Development of atherosclerotic lesions in ApoE mice

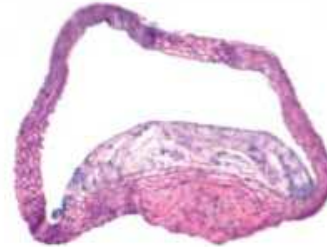
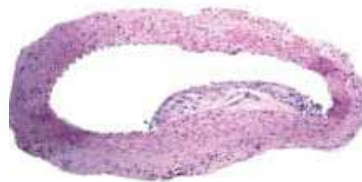


Long-term Air Pollution Exposure and Acceleration of Atherosclerosis and Vascular Inflammation in an Animal Model

6 hrs/day, 5 days/wk x 6 months
Mean levels only 15.2 $\mu\text{g}/\text{m}^3$

Filtered Air

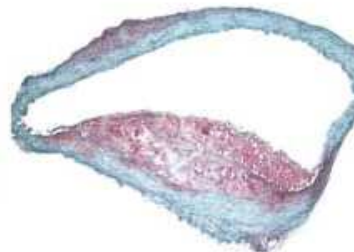
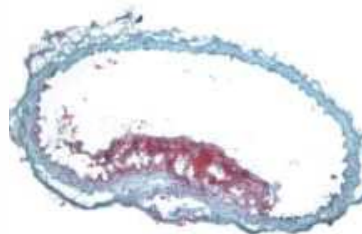
PM 2.5



Plaque area



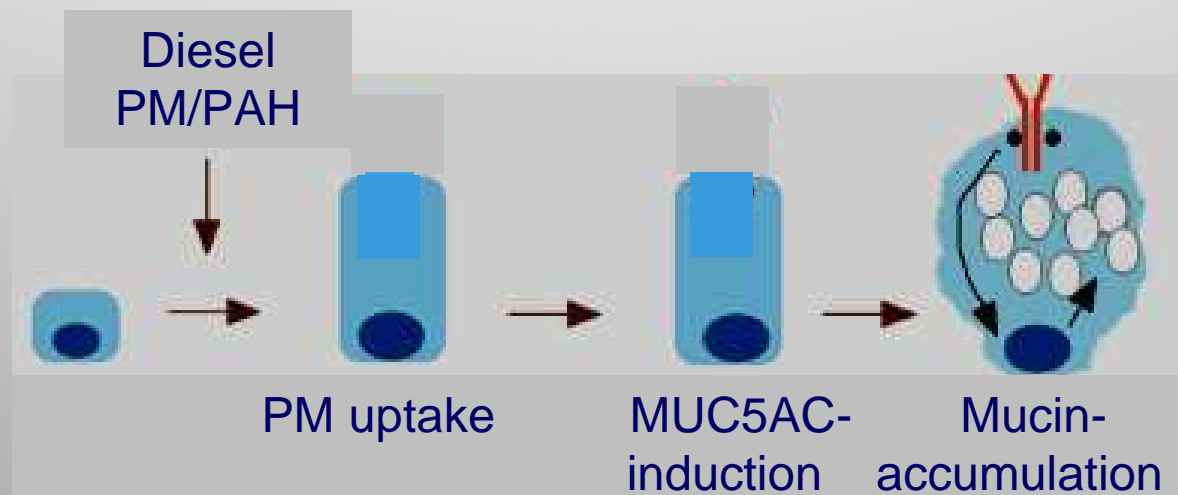
Macrophages



Lipid content

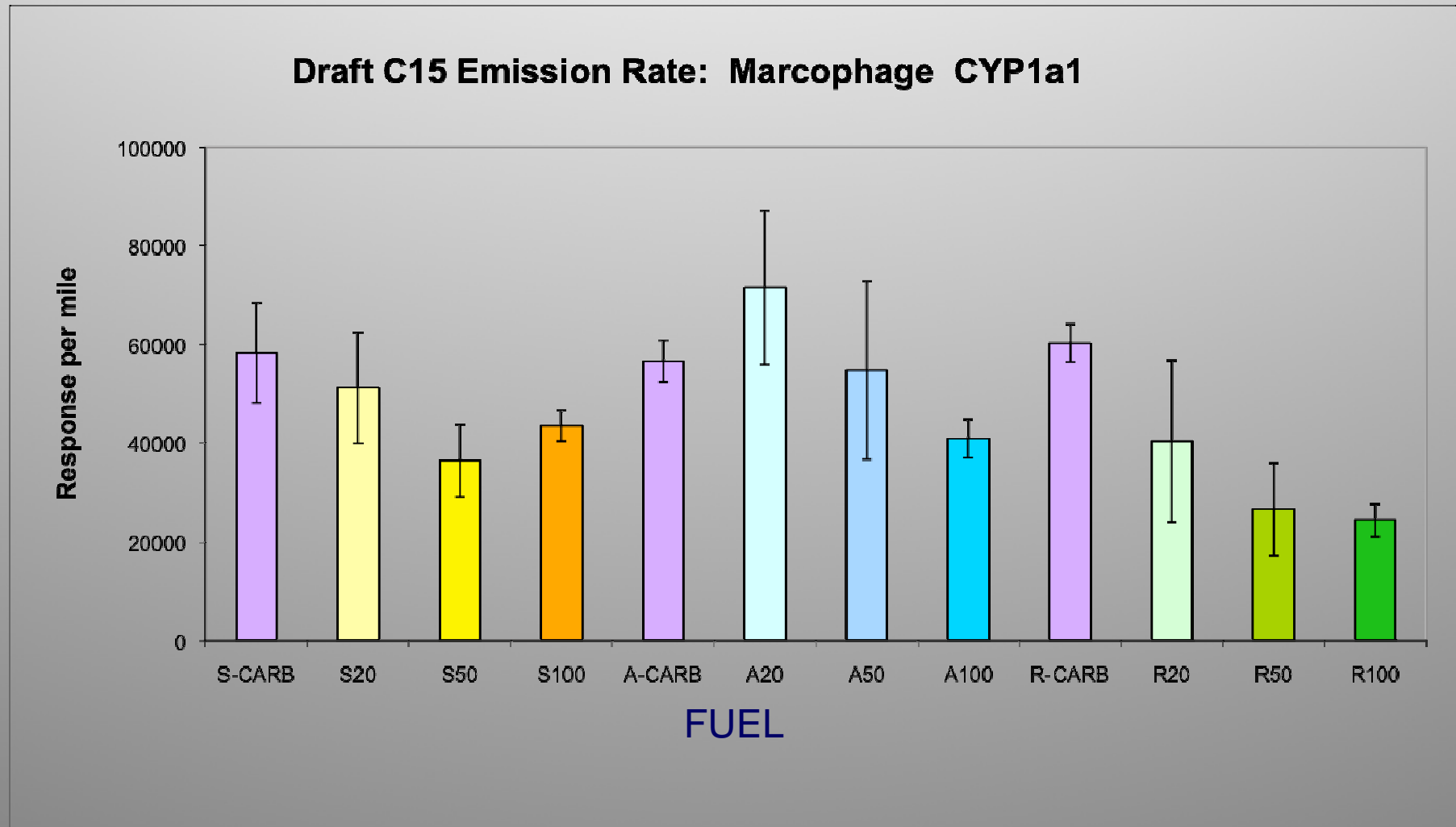
Air pollution exposed mice developed more ATHEROSCLEROSIS

Lung Clara cell model (NCIH441)

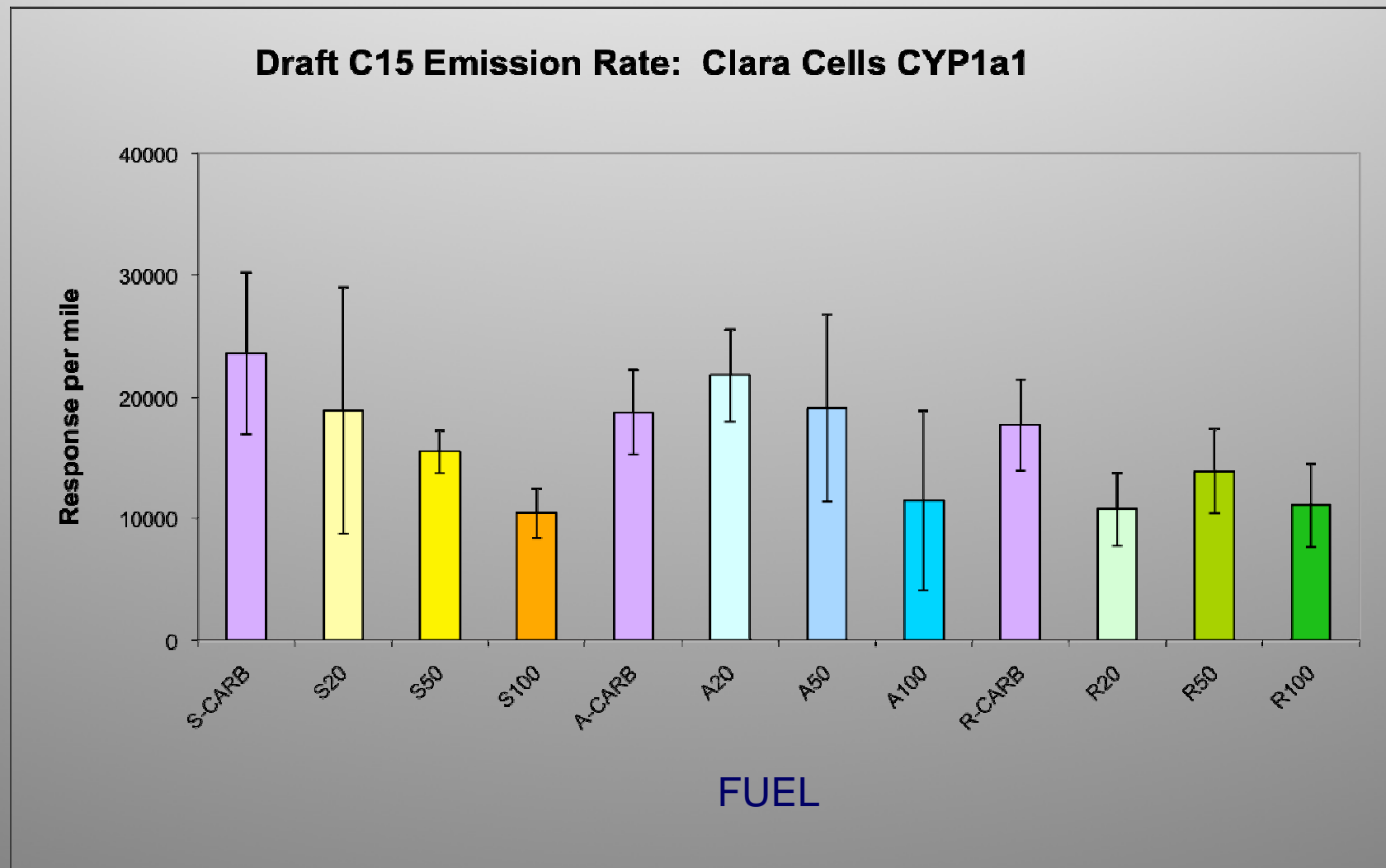


- Chronic obstructive pulmonary disease (COPD)
- Emphysema
- Asthma

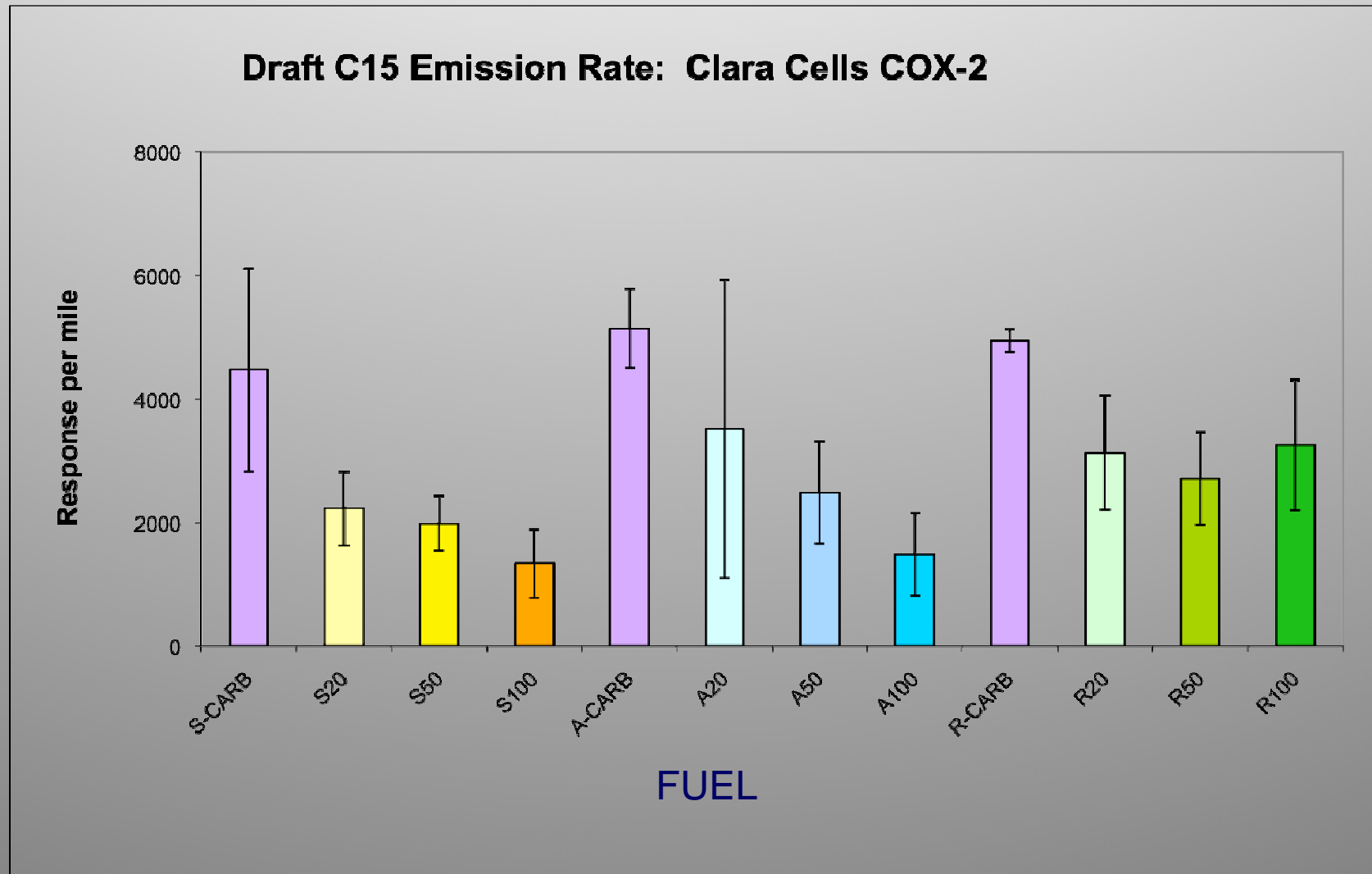
Draft C15 Emission Rate: Macrophage CYP1a1 response per mile



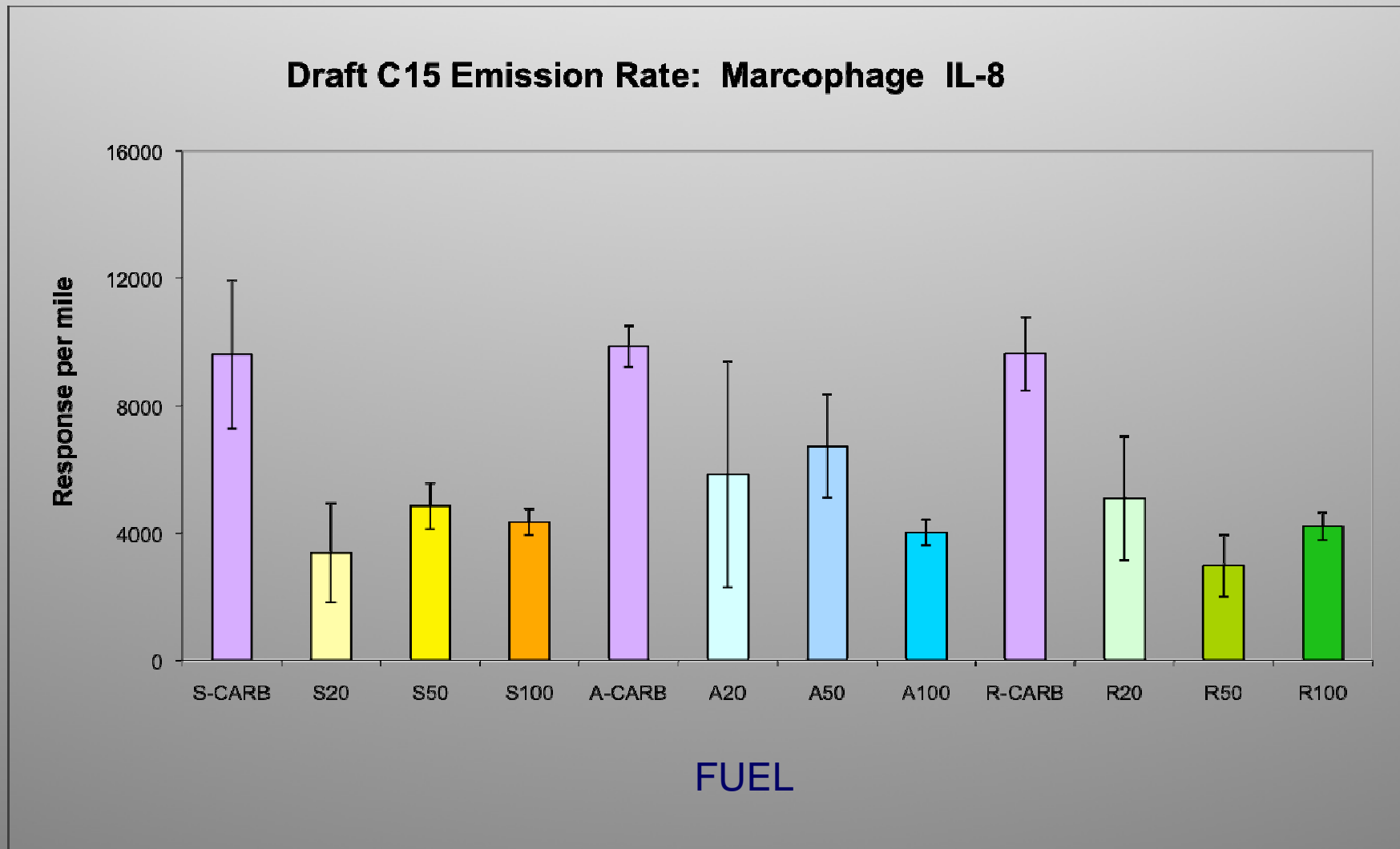
Draft C15 Emission Rate: Lung Clara cells CYP1a1 response per mile



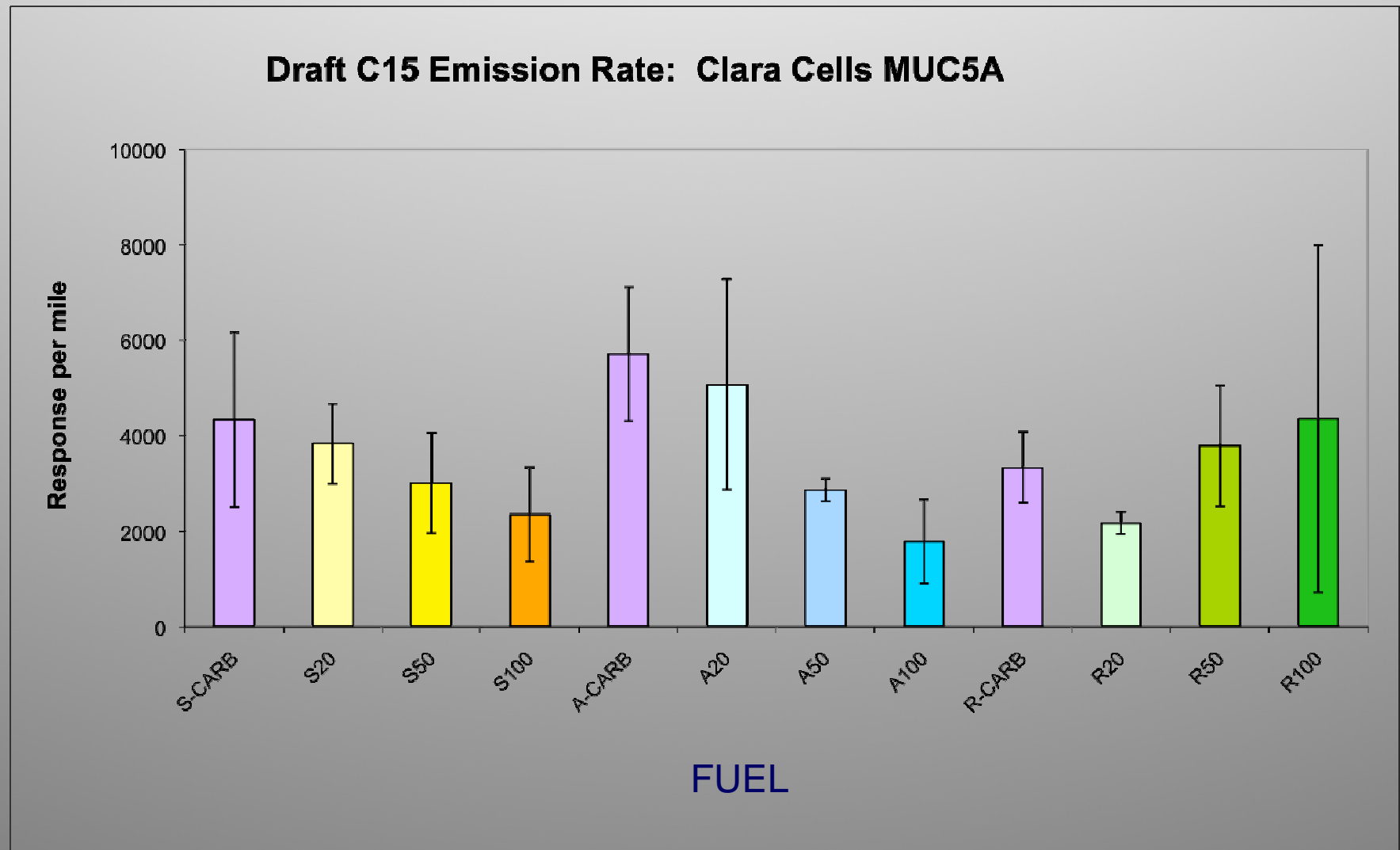
Draft C15 Emission Rate: Lung Clara cells COX-2 response per mile



Draft C15 Emission Rate: Macrophage IL-8 response per mile

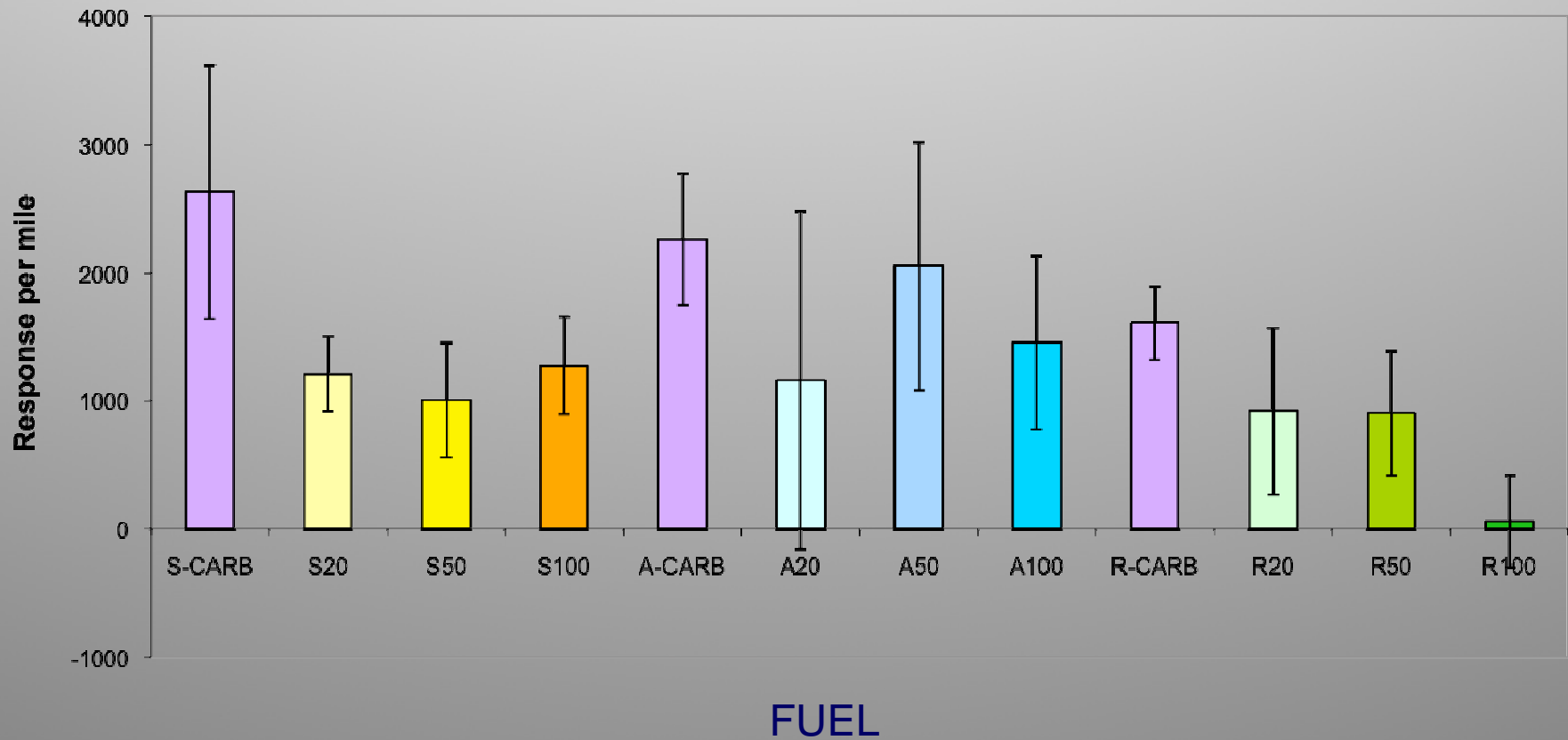


Draft C15 Emission Rate: Lung Clara cells MUC5AC response per mile



Draft C15 Emission Rate: Macrophage HO-1

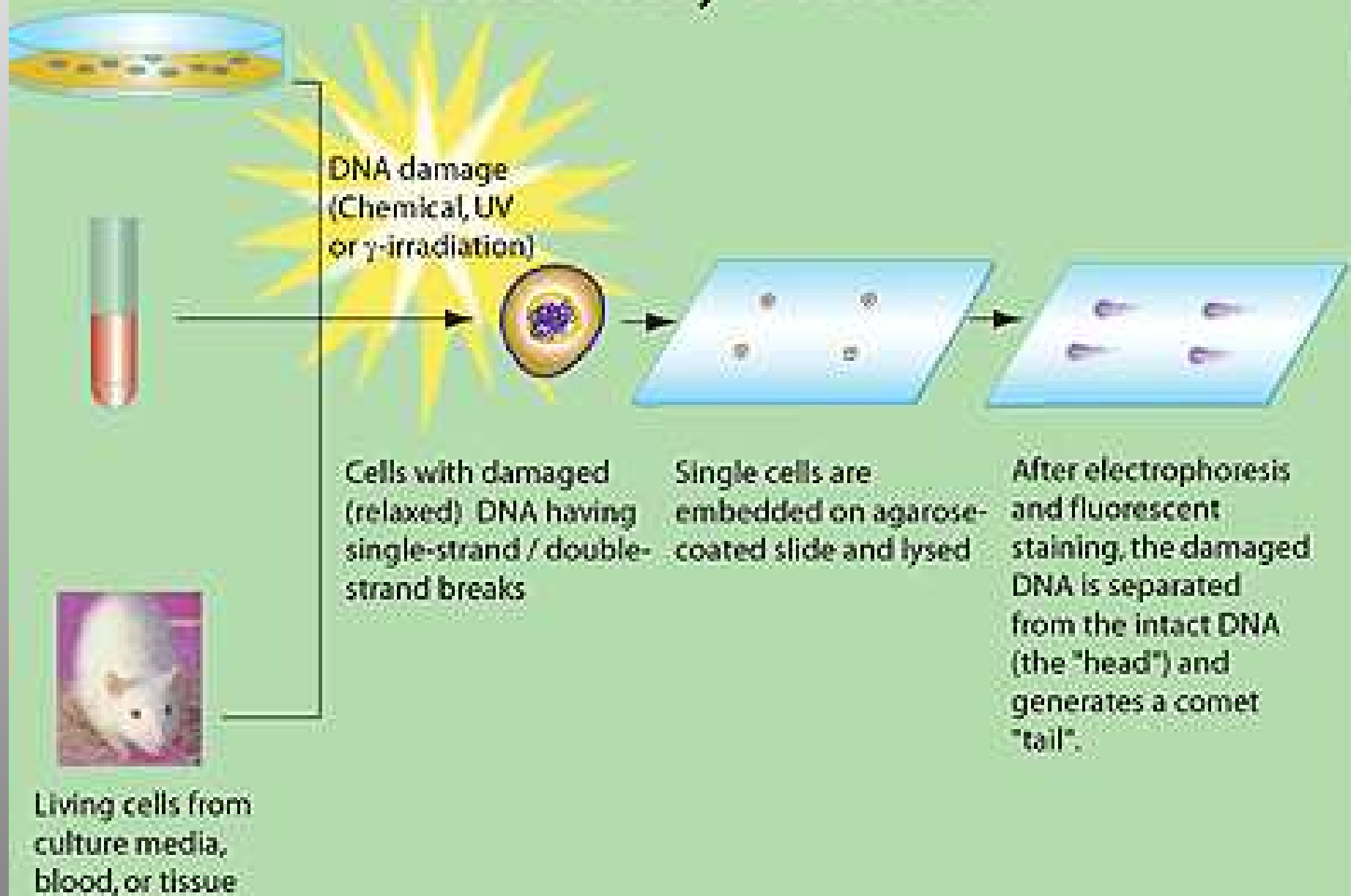
Draft C15 Emission Rate: Macrophage HO-1



Comet Assay or Single-Cell-Gel-Electrophoresis assay

- sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell
- standard technique for evaluation of DNA damage, biomonitoring genotoxicity

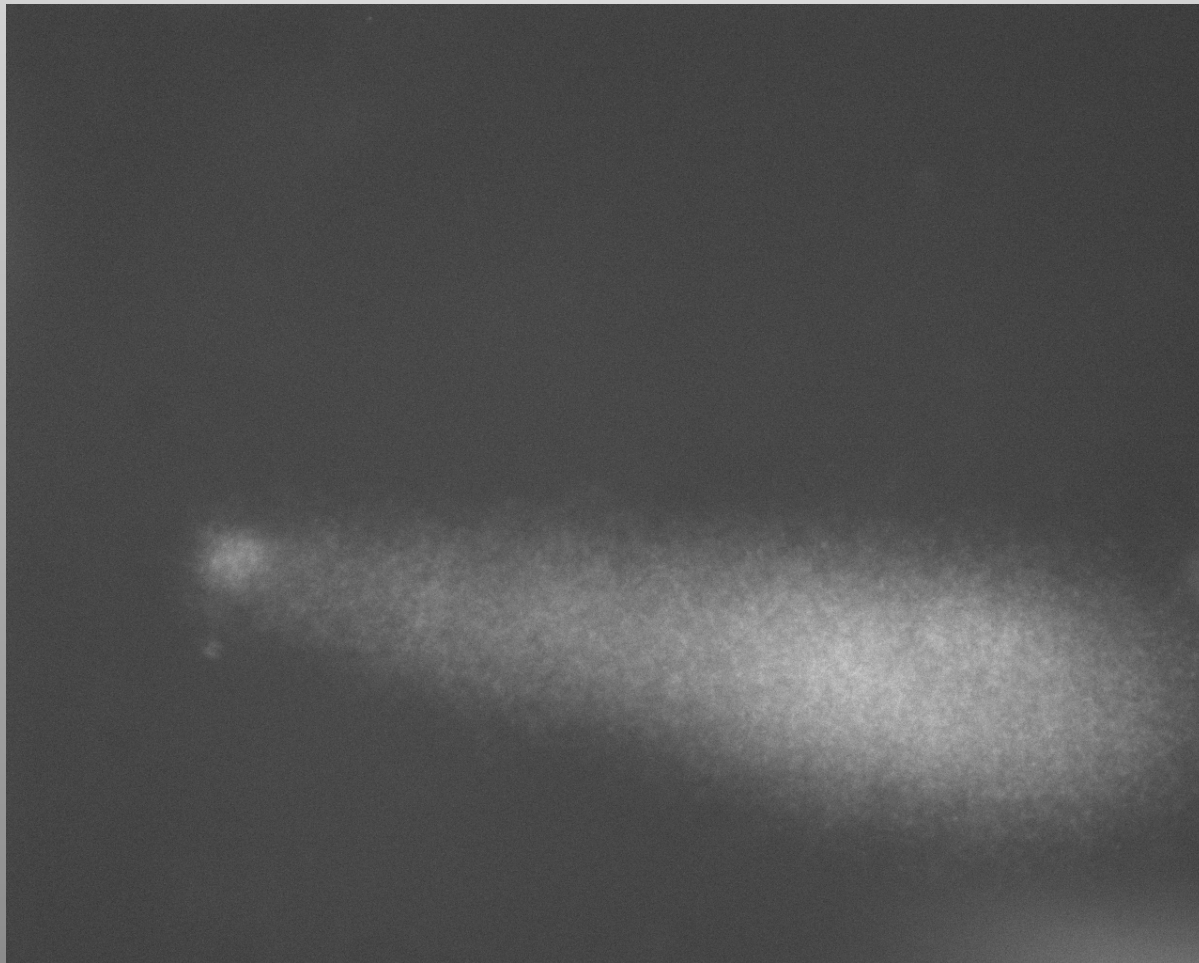
Comet Assay Overview



Comet Standard Cells 1



Comet Standard Cells 2



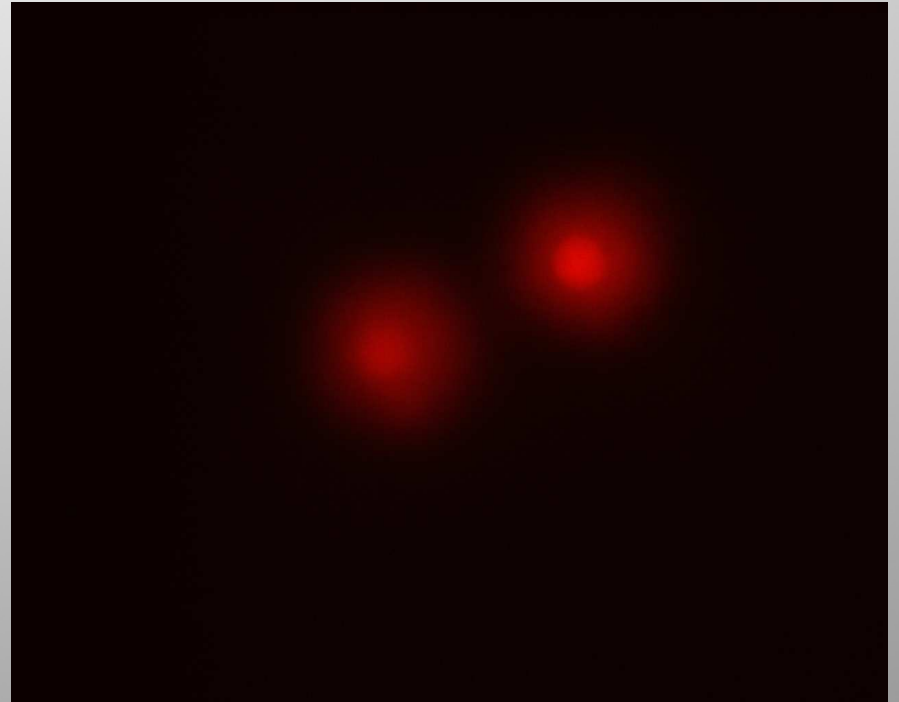
Comet Standard Cells 3



Control

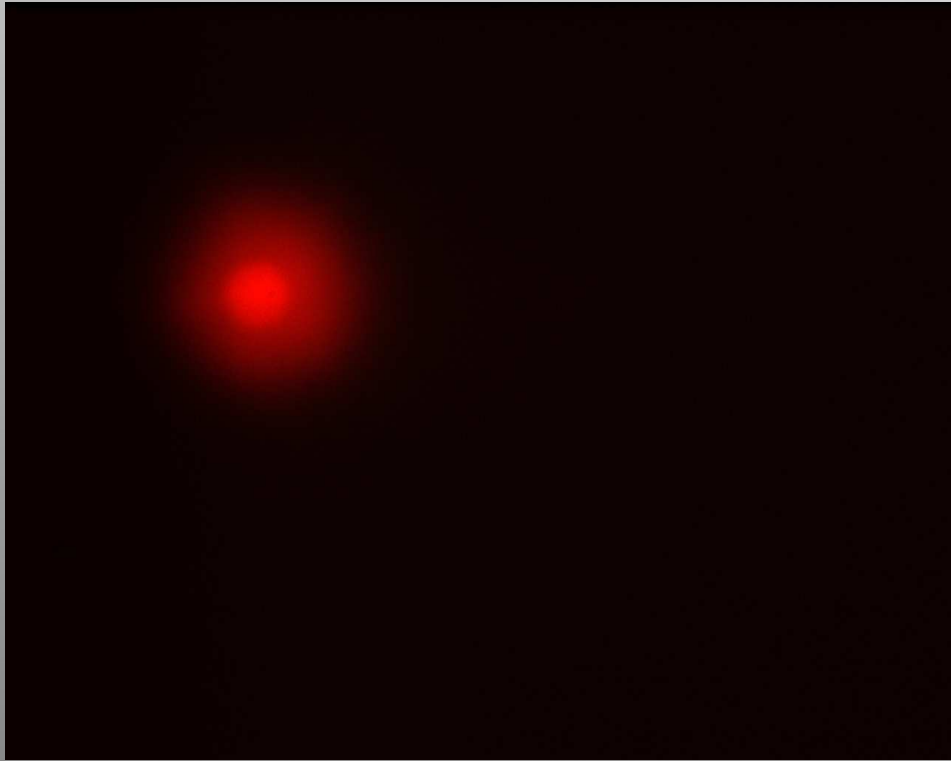


Carb



Undamaged DNA retains a highly organized association with matrix proteins in the nucleus

Soy 100

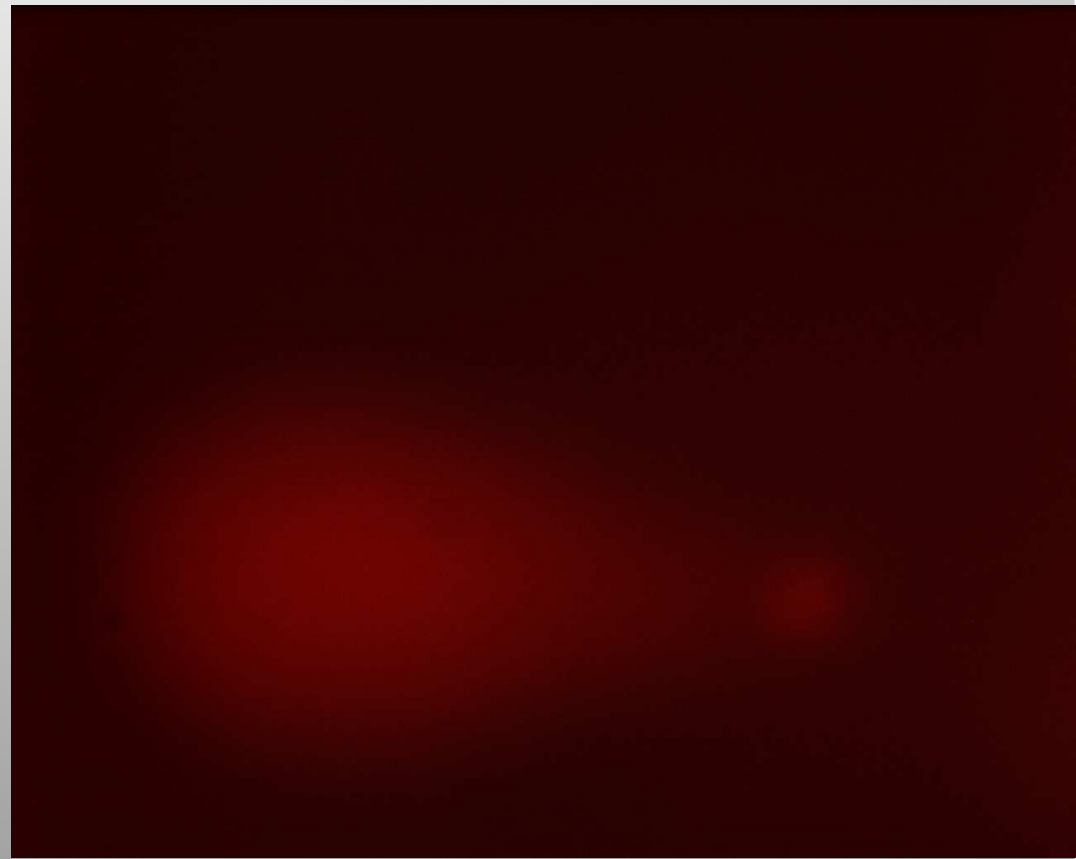
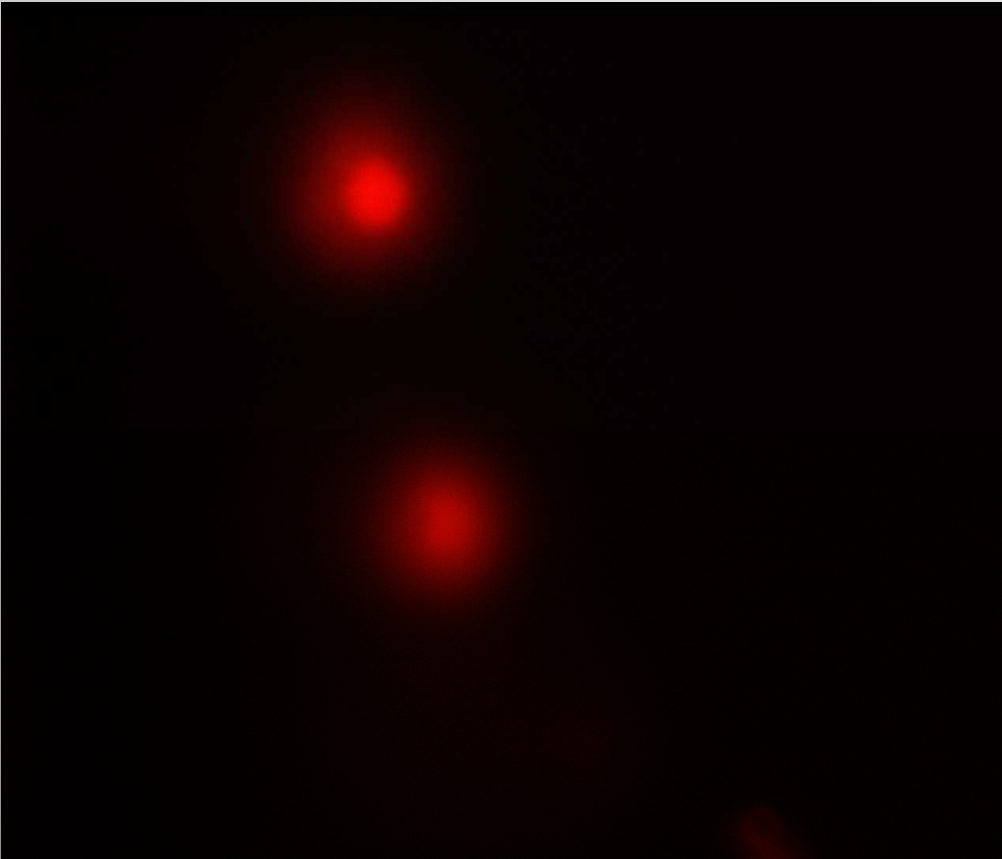


Animal 100



Renewable 100

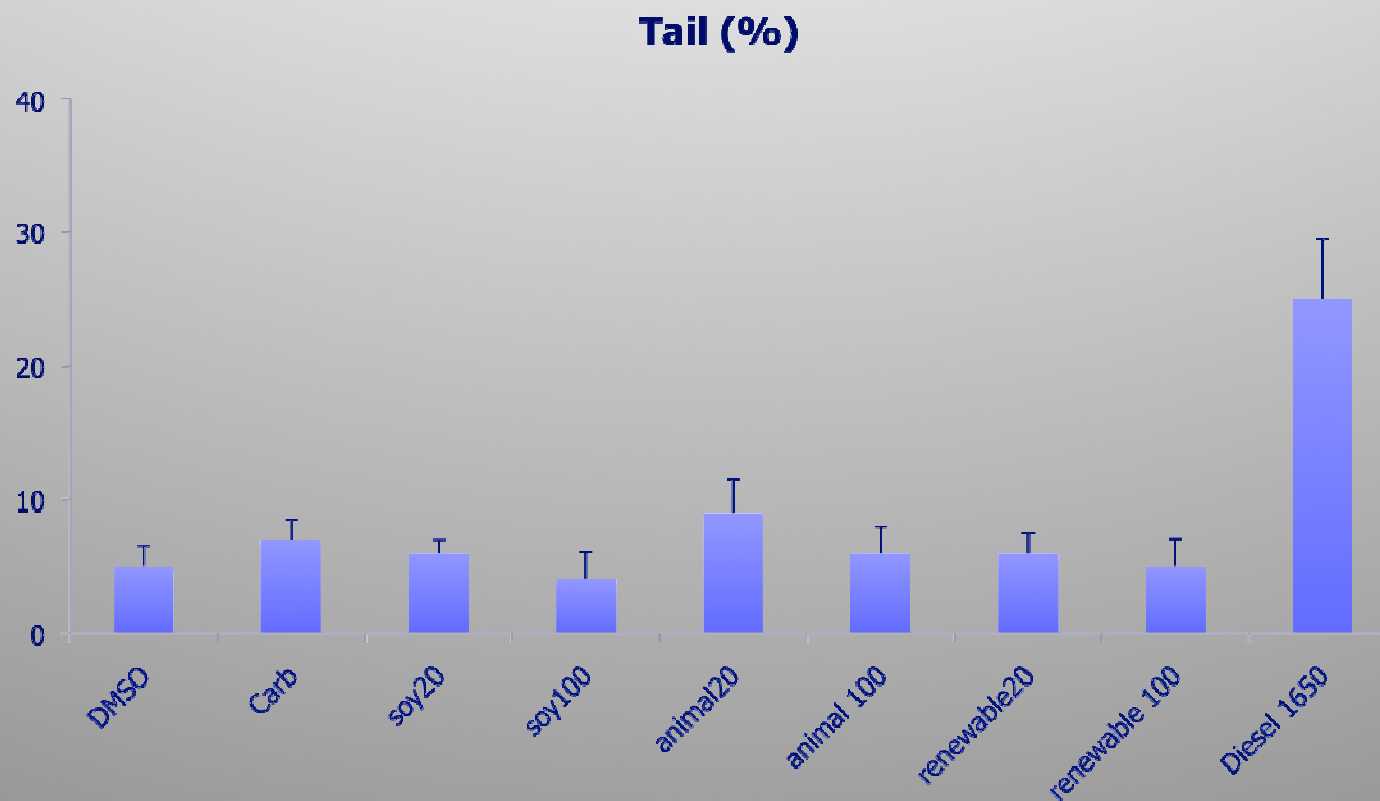
NIST SRM1650



Circular “head” corresponding to the undamaged DNA and a “tail” of damaged DNA,
the brighter and longer the tail, the higher the level of damage

NIST SRM1650

DNA damage measured by the comet assay



Percent Tail DNA was measured after 3-h treatment of U937 cells under serum-free conditions with 200 µg/ml extracts of PMs.

Summary

- Carb and Biodiesel blends induce CYP1A1 through PAHs which bind to and activate the Ah-Receptor
- Carb and Biodiesel blends induce inflammatory markers like COX-2 and IL-8 in macrophages and MUC5AC in lung Clara cell type (NCI H441)
- Effect of Biodiesel blends on inflammatory markers like COX-2 and IL-8 tend to be lower than Carb diesel
- No genotoxic effects of biodiesel blends in Comet assay

Thank you

Bob Okamoto

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Patty Lok

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Pat Wong

Fumio Matsumura